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The Study of Enzyme Hydrolysis Saccharification Process of Stems and Leaves of Banana

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Abstract

The effects of enzyme dosage, substrate concentration, pH value, reaction temperature and reaction time on enzyme hydrolysis saccharification reaction of the stems and leaves of banana were studied. The result shows that the optimized conditions are: dosage of enzyme 0.06 g/g oven dry material, substrate concentration 30g/L, pH value 5.0, reaction temperature 50℃ and reaction time 48h.Analyse the structure changes of the stems and leaves of banana before and after enzyme hydrolysis through X-ray. The result shows that the crystallinity of the stems and leaves of banana increases 10.64% after enzyme hydrolysis.

Keywords: The stems and leaves of banana; Cellulase; X-diffraction

1. Introduction

Using the stems and leaves of banana as raw materials to make methane by anaerobic fermentation. Before the anaerobic process the saccharifying enzyme hydrolysis of raw materials is the linchpin. Enzyme hydrolysis is a new technology, which mainly use cellulase to hydrolyze and ferment cellulose to generate methane [1] [2]. Enzyme hydrolysis method belongs to biological method and it has the characteristics of reacting at room temperature, low power consumption, nice selectivity of enzyme, high sugar yield, simple process and no pollution [3] [4].

Select cellulose to hydrolyze the material and confirm and optimize the conditions of enzyme hydrolysis saccharification process. The enzyme hydrolysis saccharification process are affected by many

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factors such as dosage of enzyme, substrate concentration, pH value ,reaction temperature , reaction time and enzyme inhibitor and activator. It is a complicated reaction. Use the stems and leaves of banana to study the effects of dosage of enzyme, substrate concentration, pH value, reaction temperature and reaction time on enzyme hydrolysis saccharification reaction, optimize enzyme hydrolysis process condition and increase the concentration of enzyme reducing sugar.

2. Materials and Experimental methods

2.1. Experiment Materials

(1)Materials: the stems and leaves of banana after 2% NaOH pretreatment. (2) MainReagent: Cellulase. (3)Main Equipment: Ultraviolet visible light spectro- photometer, Agilent 8453; pH plan, RHS-3C; Whole temperature vibrator, HZQ-Q; X-diffraction , D/max 2500.

2.2. Enzymatic hydrolysis

Take 2.5 g dry the stems and leaves of banana after 2% NaOH pretreatment in 250 mL grind tapered bottle. Add citric acid-phosphoric acid buffer to adjust pH value to 5.0, and add cellulase with dosage of 0.05 g/g dry raw material. Under the condition of reaction temperature 50 ℃ and reaction time 48 h ,put it in Constant Temperature Vibrator (120 r/min) to hydrolyze. Take it out and put into the refrigerator when it reaches the scheduled time. After centrifugal separation take upper clear liquid (enzymatic hydrolyzate) from the enzyme hydrolysis product.

3. Results and discussion

3.1. The effect of dosages of enzyme on enzymatic hydrolysis of the stems and leaves of banana

With the condition (substrate concentration 35g/L , pH value 5.0, reaction temperature 50℃ and reaction time 48h) study the effects of different enzyme concentration on the yield of cellulase hydrolysis reducing sugar. The results are shown in Figure1.

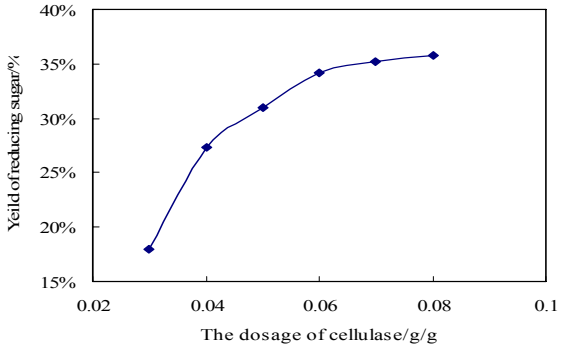

Fig. 1 The effect of dosages of cellulase on the yield of reducing sugar in the cellulase hydrolysis of the stems and leaves of banana

Figure1 shows that, with the increasing of cellulase concentration, the yield of cellulase hydrolysis reducing sugar increases. The yield of reducing sugar increases obviously when the dosage of cellulose increases under low concentration. After the dosage of cellulose reaches 0.06 g/g, the yield increases gently, which means the increase of enzyme has little contribution to the yield of reducing sugar. In the

study on enzyme hydrolysis saccharification process ,we should also consider the cost factor with the enzyme hydrolysis result. So the rational dosage of enzyme is very important. When the enzyme concentration is 0.06g/g , the effect of enzymatic hydrolysis and cost is reasonable.

3.2. The effect of substrate concentration on cellulose enzyme hydrolysis of the stems and leaves of banana

With the condition(0.05g/g, pH 5.0, reaction time 48h, temperature 50℃), the effect of different substrate concentration on the yield of banana stem leaf cellulase hydrolyzing reducing sugar was studied. The result is shown as Figure 2.

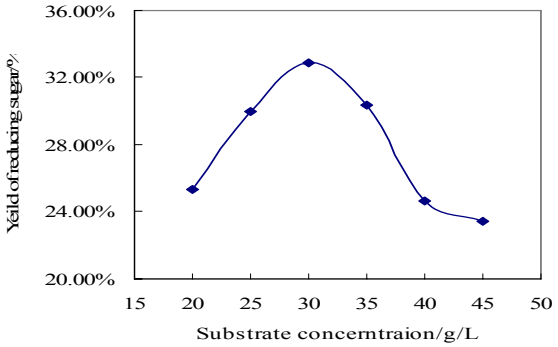

Fig. 2 The effect of substrate concentration on the yield of reducing sugar of the stems and leaves of banana

It could be found from Figure2 that the yield of banana stem leaf cellulose hydrolyzing reducing sugar shows significantly trend of increase when the substrate concentration add up from 20 g/L to 30 g/L, but when the substrate concentration reaches 30 g/L, the yield of reducing sugar hydrolyzed start to reduce and the yield of reducing sugar start to show downward trend if the substrate concentration continue to increase. Therefore, it is much better to select the substrate concentration 30 g/L for cellulase hydrolyzing banana stem.

3.3. The effect of reaction system pH value on cellulose enzyme hydrolysis of the stems and leaves of banana

With the condition of enzyme concentration 0.05g/g, the substrate concentration 35g/L, reaction time 48h, temperature 50℃, the effect of different pH value on the yield of banana stem leaf cellulase hydrolyzing reducing sugar was studied. The result is shown as Figure3.

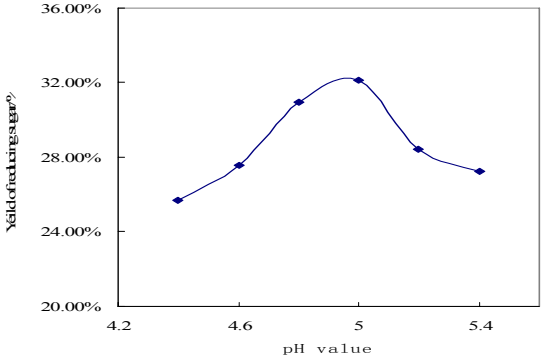

Fig. 3 The effect of system pH value on the yield of reducing sugar of the stems and leaves of banana

Figure3 shows that the reaction system pH value makes a big difference to enzyme hydrolysis of banana stem leaf. Under a certain enzyme concentration, substrate concentration, reaction time and reaction temperature, the yield of banana stem leaf enzyme hydrolyzing reducing sugar goes up with the increase of pH value within the lower range of pH value; when pH value is around to 5.0, the yield of reducing sugar reach the maximum value, the increase of pH value instead makes the yield of banana stem leaf enzyme hydrolyzing reducing sugar start to show downward trend. It means that the optimum reaction pH value is 5.0.

3.4. The effect of reaction temperature on enzymatic hydrolysis of the stems and leaves of banana

With the condition of enzyme concentration 0.05g/g, the substrate concentration 35g/L, reaction pH value5.0, reaction time 48h, the effect of different reaction temperature on the yield of banana stem leaf cellulase hydrolyzing reducing sugar was studied. The result is shown as Figure 4.

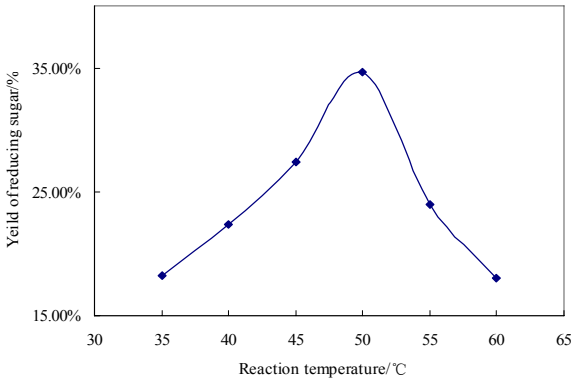

Fig. 4 The effect of reaction temperature on the yield of reducing sugar of the stems and leaves of banana

As Figure4 shown, when reaction temperature start to rise from 35℃, the yield of reducing sugar goes up with the rise of reaction temperature, and when the reaction temperature change from 35 to 50℃,the number of the enzymatic activated molecule goes up with the rise of temperature, so it makes for the process of catalyzing hydrolysis reaction, the yield of reducing sugar shows trend of increase rapidly; when reaction temperature reach to 50℃, the yield of reducing sugar turns up the maximum value, temperature continues to rise this moment, instead the yield of reducing sugar begin to show downward trend. Therefore the optimum reaction temperature is 50℃.

3.5. The effect of reaction time on enzymatic hydrolysis of the stems and leaves of banana

With the condition of enzyme concentration 0.05g/g, the substrate concentration 35g/L, reaction pH value5.0, reaction temperature 50 ℃, the effect of different reaction time on the yield of banana stem leaf cellulase hydrolyzing reducing sugar was studied. The result is shown as Figure5.

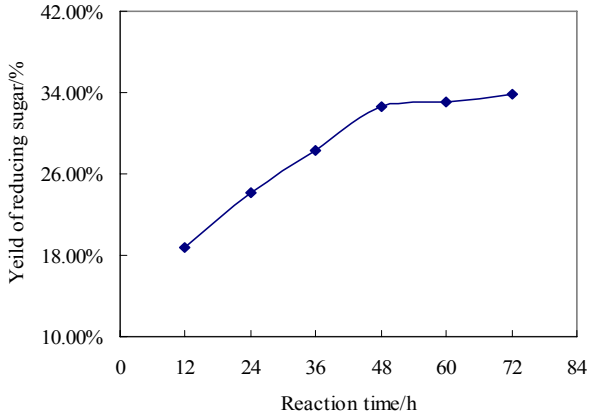

Fig. 5 The effect of reaction time on the yield of reducing sugar of the stems and leaves of banana

As Figure5 shown, at the initial stage of the enzyme hydrolysis reactions, the yield of reducing sugar continually increases and grows rapidly, it means in the stage of reaction time for12~48h, the yield of reducing sugar continually increases; yet when reaction time reach to 48h, the yield of reducing sugar increases slowly, the rate of enzyme hydrolysis reaction tends to be gentle, the yield of reducing sugar dose not increase obviously. It is because that when the enzyme hydrolysis product increases and accumulates to a certain degree, inhibitory action enhance obviously, Part of cellulose enzyme will gradually the deactivation, so hydrolysis rate becomes slow down, the yield of reducing sugar enhances slowly. So the suitable reaction time is 48h.

3.6. Crystallinity changes of the stems and leaves of banana before and after enzymatic hydrolysis

Determinate cellulose crystallinity changes before and after enzymatic hydrolysis of the stems and leaves through X-ray method. The results of crystallinity changes and the X-ray diffraction before and after enzymatic hydrolysis were shown in Figure 6 and table 1.

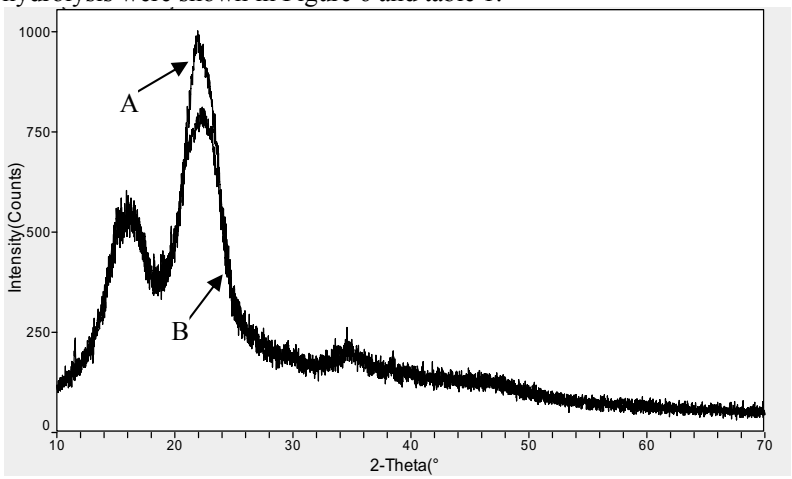

Fig. 6 The X-ray diffraction pattern before and after enzymatic hydrolysis

Table 1. Cellulose crystallinity before and after hydrolysis

sample	crystallinity /%
Before enzyme	48.03
After enzyme	58.67

Figure 6 and Table 1 show that cellulose crystallinity of the stems and leaves of banana is 48.03% before enzymatic hydrolys, and it is 58.67% after enzymatic hydrolysis, the crystallinity of the stems and leaves of banana increased 10.64%. It is because the cellulase major work on non-crystalline (amorphous regions)of the stems and leaves, hemicellulose parts and the surface part of of crystalline cellulose, for the most of crystalline cellulose ,it is difficult to adsorption, combination and enzymatic catalysis. Therefore, with the degradation of amorphous areas of cellulose macromolecules, the crystallinity of the stems and leaves of banana increased accordingly.

4. Conclusion

(1) Analysising by univariate, determine the most optimal conditions of cellulose hydrolysis of the stems and leaves of banana which is after 2% NaOH pretreatment: enzyme dosage 0.06 g / g absolutely dry raw materials, the substrate concentration 30g /L, pH value 5.0, reaction temperature 50 ℃, reaction time 48h.
(2) The X-ray diffraction analysis before and after enzyme hydrolysis of the stems and leaves, the crystallinity is 48.03% before enzyme hydrolysis and it is increased to 58.67% after enzyme hydrolysis, resulting in crystallinity of the stems and leaves of banana increased 10.64%.

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